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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/866,538	05/24/2001	Roger Y. Tsien	REGEN1530-2	4548

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HELLER EHRMAN WHITE & MCAULIFFE LLP
275 MIDDLEFIELD ROAD
MENLO PARK, CA 94025-3506

EXAMINER	
KAM, CHIH MIN	

ART UNIT	PAPER NUMBER
1653	

DATE MAILED: 12/09/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/866,538	TSIEN ET AL.	
	Examiner	Art Unit	
	Chih-Min Kam	1653	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 September 2003*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *88,93-99,102-110,128,134 and 138* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *88,93-99,102-110,128,134 and 138* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a) ☐ The translation of the foreign language provisional application has been received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *10/31/03*.
4) ☐ Interview Summary (PTO-413) Paper No(s). _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Status of the Claims

1. Claims 88, 93-99, 102-110, 128, 134 and 138 are pending.

Applicants' amendment and supplementary amendment filed August 22 and September 10, 2003 are acknowledged, and applicants' response has been fully considered. Claims 88, 93-99, 102-110, 128, 134 and 138 have been amended, and claims 89-92, 100, 101, 111-127, 129-133, 135-137 and 139-153 have been cancelled. Applicants indicate claim 89 is still pending in the Remarks (page 5 of the response), however, claim 89 has been cancelled. Therefore, claims 88, 93-99, 102-110, 128, 134 and 138 are examined.

Objection Withdrawn

2. The previous objection of claims 89, 93, 94, 95 and 98 is withdrawn in view of applicants' amendment to the claim, applicants' cancellation of the claim and applicants' response at page 7 in the amendment filed August 22, 2003.

Rejection Withdrawn

Claim Rejections - 35 USC § 101

3. The previous rejection of claims 88, 89, 93-99, 102-110, 128, 134 and 138 under 35 U.S.C.101, is withdrawn in view of applicants' cancellation of the claim, applicants' amendment of the claim, and applicants' response at page 6 in the amendment filed August 22, 2003.

Claim Rejections - 35 USC § 112

4. The previous rejection of claim 89 under 35 U.S.C.112, first paragraph, is withdrawn in view of applicants' cancellation of the claim in the amendment filed August 22, 2003.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claims 88, 93-99, 102-110, 128, 134 and 138 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a polynucleotide encoding at least one monomer of a non-oligomerizing tandem fluorescent protein, wherein the fluorescent protein comprises a first monomer of a green fluorescent protein (GFP) or a fluorescent protein related to GFP operatively linked to at least a second monomer of a GFP or a fluorescent protein related to GFP, wherein the amino acid sequence of the monomer is defined (e.g., the A206K, L221K or F223R mutant of SEQ ID NO:6 or 10), and wherein the propensity of the tandem fluorescent protein to form intermolecular oligomer is reduced or inhibited; a vector or a host cell comprising the polynucleotide; a kit comprising the polynucleotide; or, a polynucleotide encoding at least monomer of a fusion protein, wherein the fusion protein comprises the non-oligomerizing tandem fluorescent protein operatively linked to at least one polypeptide of interest, wherein the monomer and the polypeptide of interest (e.g., polyHis tag) are defined; and the polynucleotide encoding the GFP from Aequorea victoria as shown in the prior art, does not reasonably provide enablement for a polynucleotide encoding at least one monomer of a non-oligomerizing tandem fluorescent protein, wherein the protein comprises a first monomer of a GFP or a fluorescent protein related to GFP operatively linked to at least second monomer of a GFP or a fluorescent protein related to GFP, wherein the sequence of the monomer is not defined, and wherein the propensity of the tandem fluorescent protein to form intermolecular

oligomer is reduced or inhibited; a vector or a host cell comprising the polynucleotide; a kit comprising the polynucleotide; or, a polynucleotide encoding at least one monomer of a fusion protein, wherein the monomer and the polypeptide of interest are not defined. The specification does not enable a person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Claims 88, 93-99, 102-110, 128, 134 and 138 encompass a polynucleotide encoding at least one monomer of a non-oligomerizing tandem fluorescent protein, wherein the protein comprises a first and a second monomers of a GFP or a fluorescent protein related to a GFP, wherein the propensity of the tandem fluorescent protein to form intermolecular oligomer is reduced or inhibited (claims 88, 93-99 and 102); a vector (claim 128) or a host cell (claims 134) comprising the polynucleotide; a kit comprising the polynucleotide (claim 138); or a polynucleotide encoding at least one monomer of a fusion protein, wherein the fusion protein comprises the non-oligomerizing tandem fluorescent protein operatively linked to at least one polypeptide of interest (claims 103-110). The specification however, only discloses cursory conclusions without data supporting the findings, which state that the invention relates to a polynucleotide encoding a non-oligomerizing tandem fluorescent protein, which includes at least a first and a second monomers of a fluorescent protein, wherein the propensity of the tandem fluorescent protein is reduced or inhibited as compared to a monomer of the fluorescent protein, and the fluorescent protein can be a GFP, a RFP, or a fluorescent protein related to a GFP or a RFP (pages 2-3, paragraph [0008]; page 4, paragraph [0014]). There are no indicia that the present application enables the full scope in view of polynucleotides encoding at least one monomer of a non-oligomerizing tandem fluorescent protein and a fusion protein containing the

tandem fluorescent protein as discussed in the stated rejection. The present application provides no indicia and no teaching/guidance as to how the claims are enabled. The factors considered in determining whether undue experimentation is required, are summarized in In re Wands (858 F2d at 731,737, 8 USPQ2d at 1400,1404 (Fed. Cir.1988)). The factors most relevant to this rejection are the scope of the claims, the state of the prior art, the amount of direction or guidance presented, and the amount of experimentation necessary.

(1). The breath of the claims:

The breath of the claims is broad and encompasses unspecified variants regarding identities of various GFPs as monomers in the non-oligomerizing fluorescent proteins and of the polypeptide of interest in the fusion protein, which are not adequately described or demonstrated in the specification.

(2). The presence or absence of working examples:

The specification has shown the A206K, L221K and F223R mutants of SEQ ID NOs:6 and 10 are non-oligomerizing fluorescent protein (Example 1), where the replacement of the hydrophobic residues A206, L221 and F223 with residues containing positively charged side chains eliminate dimerization, while ECFP (SEQ ID NO:6) and EYFP-V68L/Q69K (SEQ ID NO:10) formed a dimer with high affinity of 113 μM; and the I125R mutant of DsRed reduced the propensity of DsRed to form tetramer, while DsRed I125K mutant was a mixture of dimer and tetramer (Example 3), and a tandem DsRed protein is formed by linking two DsRed monomers (Example 4). However, there are no other working examples indicating the claimed variants.

(3). The state of the prior art and relative skill of those in the art:

The prior art (e.g., Prasher et al.; Heim et al., 1994; Tsien *et al.*, U. S. Patent 6,140,132) disclose nucleotide and amino acid sequences of GFP variants from Aequorea Victoria; and the specification (paragraph [0059]) indicates a head-to tail, side by side dimer is formed in the crystal of GFP and its variants, and a core of hydrophobic side chains such as A206, L221 and F223 from each of the two monomers appear to be candidates for creating the contacts between monomers when GFP is in solution or expressed exogenously in cells. However, the general knowledge and level of the skill in the art do not supplement the omitted description, the specification needs to provide specific guidance on the identities of various GFPs in non-oligomerizing fluorescent proteins and polypeptides of interest in the fusion proteins containing non-oligomerizing tandem fluorescent protein to be considered enabling for variants.

(4). The amount of direction or guidance presented and the quantity of experimentation necessary:

The claims are directed to a polynucleotide encoding at least one monomer of a non-oligomerizing tandem fluorescent protein comprising at least a first and a second monomers of a GFP or a GFP-related fluorescent protein, wherein the propensity of the tandem fluorescent protein to form an intermolecular oligomer is reduced or inhibited as compared to the monomer; a vector, a host cell, or a kit comprising the polynucleotide; or a polynucleotide encoding at least one monomer of a fusion protein. The specification indicates the specific A206K, L221K and F223R mutants of SEQ ID NOs:6 and 10 are non-oligomerizing fluorescent protein (Example 1), while ECFP (SEQ ID NO:6) and EYFP-V68L/Q69K (SEQ ID NO:10) formed a dimer with high affinity of 113 μM; the I125R mutant of DsRed reduced the propensity of DsRed to form tetramer, while DsRed I125K mutant was a mixture of dimer and tetramer (Example 3); and a

tandem DsRed protein is formed by linking two DsRed monomers (Example 4). However, the specification has not identified other non-oligomerizing fluorescent proteins, nor has demonstrated the effects of reducing oligomerizing in the tandem fluorescent proteins. Moreover, there are no working examples indicating various non-oligomerizing tandem fluorescent proteins containing a GFP or a GFP-related fluorescent protein except for a tandem DsRed protein. Furthermore, the specification has not demonstrated the making and use of a fusion protein containing a defined non-oligomerizing tandem fluorescent protein and a defined target polypeptide except for a polyHis tag. Since the specification fails to provide sufficient teachings on identities of various GFP-related fluorescent proteins as the monomers of tandem fluorescent proteins, and the effects of reducing oligomerizing in these tandem fluorescent proteins, it is necessary to have additional guidance on identities of non-oligomerizing fluorescent proteins containing various GFPs as monomers and to carry out further experimentation to assess the effects of reducing oligomerizing in these tandem fluorescent proteins.

(5). Predictability or unpredictability of the art:

The specification indicates fluorescent proteins such as ECFP (SEQ ID NO:6) and EYFP-V68L/Q69K (SEQ ID NO:10) formed a dimer with high affinity of 113 μM, while the A206K, L221K and F223R mutants of SEQ ID NOs:6 and 10 are identified as non-oligomerizing fluorescent proteins (Example 1); and the DsRed I125K mutant was a mixture of dimer and tetramer, while the I125R mutant of DsRed reduced the propensity of DsRed to form tetramer (Example 3), however, it has not identified a non-oligomerizing fluorescent protein comprising

various GFPs. Since the claims encompass numerous unidentified variants, the effects of reducing oligomerizing in the tandem fluorescent proteins are unpredictable.

(6). Nature of the Invention

The scope of the claims includes many structural variants, however the specification has not identified these variants nor indicated the effects of reducing oligomerizing in the tandem fluorescent proteins. Thus, the disclosure is not enabling for reasons discussed above.

In summary, the scope of the claim is broad, while the working example does not demonstrate the claimed variants, and the teaching in the specification is limited, therefore, it is necessary to have additional guidance and to carry out further experimentation to assess the effect of reducing oligomerizing of the tandem fluorescent protein.

In response, applicants indicate the amended claims are directed to isolated polynucleotides encoding proteins comprising a GFP or GFP-related fluorescent protein and having a propensity to form intermolecular oligomers that is reduced or inhibited; and multiple examples of sequences and sequence variants of tandem fluorescent proteins are provided in the specification (pages 6-7 of the response). Applicant's response has been fully considered, however, the argument is not found persuasive because the specification only demonstrates specific fluorescent proteins such as A206K, L221K and F223R mutants of SEQ ID NOs:6 and 10 are non-oligomerizing fluorescent proteins, and the I125R mutant of DsRed reduced the propensity of DsRed to form tetramer as shown in the examples, it has not demonstrated a fluorescent protein comprising a GFP or a GFP-related fluorescent protein as monomers, where the sequence of the monomer is not defined, has non-oligomerizing effect as encompassed by the claims. Thus, the full scope of the claims is not enabled as indicated in the section above.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

6. Claims 88, 93, 99 and 102-110 are rejected under 35 U.S.C. 102(b) as anticipated by Prasher *et al.* (Gene 111, 229-233 (1992)).

Prasher *et al.* disclose the nucleotide sequence of gfp10 cDNA which encodes GFP from Aequorea victoria (pages 230-231; Fig. 2; claims 88, 93, 99 and 102-110). Since the amended claim 88 recites an isolated polynucleotide encoding at least "one monomer" of non-oligomerizing tandem fluorescent protein, and claim 103 recites an isolated polynucleotide encoding at least "one monomer" of a fusion protein, thus the reference, which teaches the Aequorea GFP nucleotide sequence, meets the criteria of claims.

7. Claims 88, 93, 99, 102-110, 128 and 134 are rejected under 35 U.S.C. 102(b) as anticipated by Heim *et al.* (Proc. Natl. Acad. Sci. USA 91, 12501-12504 (1994)).

Heim *et al.* disclose a plasmid (claim 128) containing the coding region of a clone of Aequorea victoria gfp10 cDNA (claims 88, 93, 99 and 102-110) was transformed into E. coli strain JM109 (claim 134) and high-level expression was achieved (page 12501, right column). Since the amended claim 88 recites an isolated polynucleotide encoding at least "one monomer" of non-oligomerizing tandem fluorescent protein, and claim 103 recites an isolated

polynucleotide encoding at least "one monomer" of a fusion protein, thus the reference, which teaches the Aequorea GFP nucleotide sequence, meets the criteria of claims.

8. Claims 88, 93-95, 99, 102-110, 128 and 134 are rejected under 35 U.S.C. 102(e) as anticipated by Tsien *et al.* (U. S. Patent 6,140,132, filed June 1998).

Tsien *et al.* disclose the nucleotide and amino acid sequences of GFP variants from Aequorea victoria (column 4, lines 54-56, Table 1-8; claims 88, 93, 99 and 102-110), e.g., EYFP-V68L/Q69K (Table 7, SEQ ID NO:9) and ECFP mutant (Table 5, Example 1; claims 94-95). The reference also teaches using the nucleic acids in construction of expression vector and transfecting into host cells for expression of the protein (columns 11-15; claims 128 and 134). Since the amended claim 88 recites an isolated polynucleotide encoding at least "one monomer" of non-oligomerizing tandem fluorescent protein, and claim 103 recites an isolated polynucleotide encoding at least "one monomer" of a fusion protein, thus the reference, which teaches the Aequorea GFP nucleotide sequence, meets the criteria of claims.

Conclusion

9. No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period

will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Chih-Min Kam whose telephone number is (703) 308-9437. The examiner can normally be reached on 8.00-4:30, Mon-Fri.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached on (703) 308-2923. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-0294 for regular communications and (703) 308-4227 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Chih-Min Kam, Ph. D. CMK
Patent Examiner

December 04, 2003

CHRISTOPHER S. F. LOW
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600